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Culture of mouse pancreatic islets in different glucose concentrations modifies B cell sensitivity to streptozotocin

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Summary. There have previously been divergent data published regarding the effects of glucose on the diabetogenic effects of streptozotocin. In order to further explore this issue, two separate sets of experiments were performed. In the first, mouse pancreatic islets were maintained in culture for 3 days at different glucose concentrations (5.6, 11.1 and 28 mmol/l) and then exposed to streptozotocin. After another 3 days in culture at 11.1 mmol/l glucose, the B cell function was evaluated by measurement of glucose-stimulated insulin release, the number of islets recovered after culture, and the islet DNA and insulin contents. In the second group of experiments islets were first maintained in culture at 11.1 mmol/l glucose, then treated with streptozotocin and subsequently cultured for 6 days at the different glucose concentrations given above. It was found that islets maintained in a medium containing 28 mmol/l glucose before or after streptozotocin exposure showed less signs of damage than islets cultured in 11.1 mmol/l glucose. A similar, but less pronounced, decreased sensitivity to streptozotocin was found in islets precultured in 5.6 mmol/l glucose, in comparison with those islets cultured in 11.1 mmol/l glucose. Culture at 5.6 mmol/l glucose just after streptozotocin treatment did not induce any improvement in islet survival or function. It is suggested that the increased damage induced by streptozotocin to islets precultured at 11.1 mmol/l glucose, in comparison with 5.6 mmol/l glucose, can be related to the fact that an increased metabolic activity of B cells render them more susceptible to the toxin. The improved preservation of islets cultured at 28 mmol/l glucose before or after streptozotocin treatment may reflect an additional effect of glucose, i.e. activation of defense mechanisms in the B cells against cytotoxins.

Key words: Glucose, streptozotocin, pancreatic islets, insulin secretion, tissue culture.

Insulin-dependent diabetes mellitus is caused by a marked reduction in the number of pancreatic B cells, probably as the result of an interaction between environmental factors and a genetic predisposition, leading to an autoimmune destruction of the B cells [1]. For reasons that are still essentially unclear, the susceptibility of the B cell to damage may vary between individuals.

Streptozotocin (SZ) has been widely used as a drug for causing islet injury and for the induction of experimental diabetes [2]. The compound has a direct cytotoxic effect, but in lower doses it may also trigger an autoimmune process against the B cells [3].

It has previously been shown that B cells from rats adapted to a carbohydrate-free, high protein diet, show a decreased sensitivity to SZ, in vivo [4] and in vitro [5]. However, the exact effect of carbohydrates, and glucose in particular, on the diabetogenic effects of SZ remains controversial. In vivo studies in rodents, involving acute infusions of glucose immediately, or within minutes, prior to SZ injection showed no protection [6-8] or potentiation of the diabetogenic action of SZ by glucose [9-12]. In vitro studies, involving preincubation of rat or mouse pancreatic islets with various glucose concentrations, followed by exposure to SZ and the measurement of islet proinsulin biosynthesis or insulin release, showed either a protective effect [13], a lack of effect [14] or even a potentiation of the cytotoxicity of SZ by glucose [15].

The aim of the present study was to further investigate the effects of glucose on the cytotoxic effects of SZ on B cells, in order to examine in vitro conditions mediating either an increased susceptibility or resistance to the toxin. Mouse pancreatic islets were thus maintained in culture in different glucose concentrations (5.6, 11.1 and 28 mmol/l) for 3 days and then exposed to SZ. The B cell sensitivity to SZ was estimated after a further 3 days in tissue culture at 11.1 mmol/l

glucose, by measurement of glucose-stimulated insulin release, the number of islets recovered after culture and the islet DNA and insulin contents. In a separate set of experiments the effects of different glucose concentrations after exposure to SZ were also evaluated. For this purpose the islets were first maintained in culture for 5 days at 11.1 mmol/l glucose, then treated with SZ and subsequently cultured for six days at the different glucose concentrations given above.

Materials and methods

Chemicals

Streptozotocin (SZ) was a gift of Dr. W. E. Dulin, Upjohn Company, Kalamazoo, MI, USA. Collagenase produced from Clostridium histolyticum was obtained from Boehringer-Mannheim, Mannheim, FRG, and bovine albumin (fraction V) was from Miles Laboratories, Slough, UK. Hanks' solution and calf serum were supplied by Statens Bakteriologiska Laboratorium, Stockholm, Sweden. Benzylpenicillin was from Astra Läkemedel, Södertälje, Sweden, and streptomycin was obtained from Glaxo Laboratories, Greenford, UK. Culture medium RPMI 1640 was from Flow Laboratories, Irvine, UK. Ficoll 400 was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Antibovine insulin serum was from Miles-Yeda, Rehovot, Israel. Crystalline mouse insulin and ^{125}I-labelled insulin were provided by Novo, Copenhagen, Denmark. Hepes was supplied by Sigma Chemicals, St Louis, MO, USA. Other chemicals of analytical grade were obtained from E. Merck, Darmstadt, FRG.

Islet preparation and culture

Pancreatic tissue from male NMRI mice (Anticimex, Stockholm, Sweden), which had been starved overnight, was used. Islets were isolated from the pancreas by a collagenase digestion technique [16] and subsequently picked free from exocrine tissue by means of a braking pipette. The islets were maintained free-floating in tissue culture, at 37 °C in air + 5% CO_2, for 3 days before exposure to SZ. The culture medium was RPMI 1640 containing 10% calf serum, benzylpenicillin (100 U/ml), streptomycin (0.1 mg/ml) and glucose at three different concentrations, i.e. 5.6, 11.1 or 28 mmol/l. The culture medium was changed every 48 h. For the series of experiments where the effects of glucose were studied after SZ exposure, the islets were isolated with the aid of Ficoll gradients [17] from the collagenase-digested pancreata. The islets were maintained in culture for 5 days in RPMI 1640 containing 11.1 mmol/l glucose as described above. On the fifth day, the islets were exposed to SZ and then transferred to culture for another 6 days at three different glucose concentrations, i.e. 5.6, 11.1 and 28 mmol/l.

Treatment of islets with streptozotocin

The islets were treated with SZ in vitro according to a previously described procedure [15]. In the first series of experiments, groups of 45 islets each were transferred to plastic dishes containing 1 ml of a bicarbonate buffer [18] supplemented with 10 mmol/l Hepes and albumin (2 mg/ml) (hereafter designated as KRBH buffer) and glucose, at the same concentrations as in the previous culture period (5.6, 11.1 or 28 mmol/l). The islets were first preincubated for 30 min at 37 °C (air + 5% CO_2). SZ was dissolved, within 1 min before use, in cold citrate buffer (10 mmol/l, pH 4.5) and 5–10 µl of this solution were then added to the islets to obtain the required SZ concentrations (1.1 or 2.2 mmol/l). The SZ incubations were performed for 30 min at 37 °C (air + 5% CO_2), and terminated by the addition of 3 ml of KRBH with 5.6 mmol/l glucose. The islets were then immediately transferred, in groups of 40, for culture for a subsequent 3 days in medium RPMI 1640, containing 11.1 mmol/l glucose. In each experiment, corresponding control islets were treated similarly with citrate buffer (10 µl).

In a second set of experiments, the islets in groups of 50 were incubated with 1.8 mmol/l SZ or citrate buffer as described above and subsequently cultured for another 6 days at either 5.6, 11.1 or 28 mmol/l glucose. A lower concentration of SZ was used in these experiments since a new batch of the toxin was found to be more potent than the one used for the initial experiments.

Islet recovery, insulin release and insulin and DNA content

The number of islets were recounted, using a stereomicroscope, on the last day of culture after SZ exposure, and all the remaining islets were recovered and utilised for the determinations of insulin release and insulin and DNA contents. For the insulin release experiments, these islets were divided into triplicate groups of 10–14 islets each and placed in sealed glass vials [19] containing 0.25 ml of KRBH. During the first hour of incubation at 37 °C (O_2:CO_2; 95:5) the KRBH medium contained 1.67 mmol/l glucose. The medium was then gently removed and replaced by 0.25 ml of KRBH supplemented with 16.7 mmol/l glucose and the incubation continued for a second hour. The insulin concentration in the incubation medium was determined by radioimmunoassay [20], using mouse crystalline insulin as standard and ^{125}I-labelled insulin as the tracer. In each experimental group the insulin secretion was calculated as a mean from the three incubation vials.

After the insulin release experiments, the islets were pooled and disrupted by sonication in 0.2 ml redistilled water. A 50 µl aliquot of the aqueous homogenate was mixed with 125 µl of acid-ethanol (0.18 M HCl in 96% (vol/vol) ethanol) and the insulin extracted overnight at 4 °C. DNA was measured in another fraction of the water homogenate, according to the method described by Kissane and Robins [21] and Hinegardner [22].

Statistical analysis

Means ± SEM were calculated. Groups of SZ-treated islets were compared with their corresponding control group using Student's unpaired or paired t-tests. For multiple comparisons between the groups of SZ-treated islets, analysis of variance was performed.

Results

Effects of streptozotocin on islets precultured at different glucose concentrations

In the non-SZ-treated groups (controls) preculture with different concentrations of glucose did not affect the recovery of islets after 72 h in culture (Table 1). SZ at a concentration of 1.1 mmol/l did not induce any decrease in the islet number after culture (data not shown). However, at a concentration of 2.2 mmol/l, SZ induced a decrease in the number of islets retrieved after previous culture at 11.1 mmol/l glucose, whereas no loss of islets was observed after preculture at 5.6 or 28 mmol/l glucose (Table 1). These findings were also confirmed by a lower total islet DNA recovery (expressed as % of the controls) only in the group of islets precultured with 11.1 mmol/l glucose. There was no difference in the DNA content of individual islets (ng/

Table 1. Islet number and DNA content in islets exposed to streptozotocin (SZ) after preculture at different glucose concentrations

Preculture glucose (mmol/l)	Treatment SZ (mmol/l)	Islet recovery (% of original number)	DNA content		
			ng/10 islets	Total islet DNA recovery (ng)	% of the controls
5.6	0	94.2 ± 1.6	373 ± 26	1407 ± 106	-
5.6	2.2	96.7 ± 1.2	354 ± 20	1374 ± 85	99.4 ± 5.3
11.1	0	94.4 ± 2.6	368 ± 23	1394 ± 100	-
11.1	2.2	77.8 ± 2.1[a]	351 ± 37	1085 ± 109	78.0 ± 6.1[b]
28.0	0	94.7 ± 1.4	347 ± 30	1312 ± 111	-
28.0	2.2	90.0 ± 2.1	332 ± 18	1199 ± 79	93.6 ± 4.6

Islets were precultured for 3 days in medium RPMI 1640 supplemented with 5.6, 11.1 or 28 mmol/l glucose and then exposed to SZ (2.2 mmol/l). Islets were counted again, and the DNA content determined after another 3 days in culture at 11.1 mmol/l glucose. The % of the controls was determined in each individual experiment by calculating the percentage of total islet DNA content of the SZ exposed groups compared with their corresponding controls. Values are means ± SEM for 9 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the corresponding control islets cultured at the same glucose concentrations are: [a] $p < 0.001$ using unpaired Student's t-test and [b] $p < 0.01$ using paired Student's t-test

Table 2. Insulin content of islets exposed to streptozotocin (SZ) after preculture at different glucose concentrations

Preculture glucose (mmol/l)	Treatment SZ (mmol/l)	Insulin content		
		ng/10 islets	Total islet insulin recovery (ng)	% of the controls
5.6	0	489 ± 27	1836 ± 99	-
5.6	2.2	261 ± 30[a]	1004 ± 106[a]	54.9 ± 5.2[b]
11.1	0	692 ± 79	2654 ± 309	-
11.1	2.2	206 ± 21[a]	629 ± 65[a]	29.1 ± 4.2[b]
28.0	0	559 ± 43	2117 ± 170	-
28.0	2.2	447 ± 74	1610 ± 260	74.6 ± 13.3

Islets were exposed to SZ after preculture in different glucose concentrations, as described in Table 1. The % of the controls was determined in each individual experiment by calculating the percentage of total insulin recovered from the SZ exposed islets compared with their corresponding controls. Values are means ± SEM for 8 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the corresponding control islets cultured at the same glucose concentrations are: [a] $p < 0.001$ using unpaired Student's t-test and [b] $p < 0.001$ using paired Student's t-test

10 islets) in any of the groups, irrespective of whether the islets had been exposed to SZ or not.

After treatment with 2.2 mmol/l SZ there was a decline in the islet insulin content and total insulin recovered in islets previously maintained in 5.6 or 11.1 mmol/l glucose, compared with the control islets (Table 2). This decrease was not observed in islets precultured with 28 mmol/l glucose before SZ exposure. Furthermore, these islets showed greater insulin contents per islet, compared to islets previously maintained in 11.1 mmol/l glucose ($p < 0.005$).

Three days after treatment with 1.1 mmol/l SZ, the insulin release in response to 16.7 mmol/l glucose was not diminished in any of the groups, although there was a trend towards lower values in the group of islets previously cultured with 11.1 mmol/l glucose (data not shown). There were no differences in the basal insulin release, at 1.67 mmol/l glucose, of the islets exposed to 2.2 mmol/l SZ as compared to the controls (Table 3). However, at 16.7 mmol/l glucose exposure, the insulin release was diminished in all SZ treated groups in comparison with the islets not exposed to SZ. This decrease in insulin release, in comparison to the corresponding control islets, was less pronounced in islets precultured in 28 mmol/l glucose. These islets also released more insulin after a glucose challenge than islets previously maintained in either 5.6 or 11.1 mmol/l glucose before the SZ treatment ($p < 0.001$).

Effects of different glucose concentrations in culture after streptozotocin exposure

In this series of experiments, which were performed 8 months after the initial experiments, islets were separated by Ficoll gradients after the collagenase digestion of the pancreas. This procedure allows identification and isolation of islets of smaller size. It is likely that the low DNA content obtained also in the control islets reflects this methodological alteration (Table 4). Islets cultured at 5.6 or 11.1 mmol/l glucose after the SZ treatment showed lowered DNA contents compared to their control groups, however, this was not the case for islets cultured at 28 mmol/l glucose after SZ. The control islets cultured at 5.6, 11.1 or 28 mmol/l glucose all showed an islet retrieval around 90% (Table 4). In comparison to islets maintained at either 5.6 or 11.1 mmol/l glucose after SZ, there was an increased number of islets recovered in the group of islets kept at 28 mmol/l glucose ($p < 0.02$ and $p < 0.05$, respectively).

There was a lowering in the insulin content compared to the controls in the groups of islets exposed to SZ and subsequently cultured at 11.1 or 28 mmol/l glucose (Table 5). This was not observed in islets cultured at 5.6 mmol/l glucose after SZ, but it should also be noted that the control islets cultured at 5.6 mmol/l glucose exhibited a lower insulin content compared to the other groups of control islets not exposed to SZ. The total islet insulin recovery, expressed as percentage of the controls, was diminished to about the same extent in all groups of SZ treated islets.

Table 3. Glucose-stimulated insulin release in islets exposed to streptozotocin (SZ) after preculture in different glucose concentrations

Preculture glucose (mmol/l)	Treatment SZ (mmol/l)	Insulin release		
		1.67 mmol/l glucose (ng/10 islets × 60 min)	16.7 mmol/l glucose (ng/10 islets × 60 min)	% of the controls
5.6	0	1.0 ± 0.3	23.1 ± 2.3	-
5.6	2.2	0.9 ± 0.5	6.6 ± 1.4[b]	28.4 ± 4.7[d]
11.1	0	0.9 ± 0.3	28.5 ± 2.8	-
11.1	2.2	0.6 ± 0.1	6.5 ± 1.1[b]	26.0 ± 4.0[d]
28.0	0	0.6 ± 0.1	22.4 ± 2.5	-
28.0	2.2	0.6 ± 0.1	14.6 ± 1.9[a]	69.0 ± 8.8[c]

Islets were exposed to SZ after preculture in different glucose concentrations, and subsequently maintained in culture as described in Table 1. Islet insulin release was measured by incubating islets in groups of 10-14, as described in Materials and Methods. The % of the controls was determined in each individual experiment by calculating the percentage of insulin release at 16.7 mmol/l glucose of the SZ exposed groups compared with their controls. Values are means ± SEM for 9 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the corresponding control islets cultured at the same glucose concentrations are: [a] $p < 0.05$; [b] $p < 0.001$, using unpaired Student's t-test and [c] $p < 0.01$; [d] $p < 0.001$, using paired Student's t-test

Table 4. Islet number and DNA content in islets exposed to streptozotocin (SZ) and then postcultured at different glucose concentrations

Postculture glucose (mmol/l)	Treatment SZ (mmol/l)	Islet recovery (% of original number)	DNA content		
			ng/10 islets	Total islet DNA recovery (ng)	% of the controls
5.6	0	94.0 ± 1.8	107 ± 15	500 ± 62	-
5.6	1.8	51.7 ± 5.8[c]	50 ± 7[a]	130 ± 30[b]	26.4 ± 5.1[e]
11.1	0	86.0 ± 2.9	118 ± 10	476 ± 43	-
11.1	1.8	59.7 ± 5.0[b]	67 ± 9[b]	206 ± 37[b]	42.1 ± 5.5[e]
28.0	0	88.8 ± 2.6	124 ± 12	560 ± 61	-
28.0	1.8	75.2 ± 4.4	95 ± 20	365 ± 82	65 ± 12[d]

Islets were precultured in medium RPMI 1640 (11.1 mmol/l glucose) and then exposed to 1.8 mmol/l SZ. The islets were subsequently cultured in medium RPMI 1640 supplemented with either 5.6, 11.1 or 28 mmol/l glucose. After 6 days the islets were counted again and the DNA content determined. The % of the controls was determined in each individual experiment by calculating the percentage of total islet DNA content of the SZ exposed groups compared with their corresponding controls. Values are means ± SEM for 5-6 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the corresponding control islets, postcultured at the same glucose concentration, are: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$, using unpaired Student's t-test and [d] $p < 0.05$; [e] $p < 0.001$, using paired Student's t-test

Table 5. Insulin content in islets exposed to streptozotocin (SZ) and then postcultured at different glucose concentrations

Postculture glucose (mmol/l)	Treatment SZ (mmol/l)	Insulin content		
		ng/10 islets	Total islet insulin recovery (ng)	% of the controls
5.6	0	213 ± 74	997 ± 352	-
5.6	1.8	110 ± 64	236 ± 106	23.1 ± 3.1[e]
11.1	0	732 ± 25	3047 ± 57	-
11.1	1.8	287 ± 84[b]	849 ± 230[c]	27.8 ± 7.6[e]
28.0	0	565 ± 68	2515 ± 352	-
28.0	1.8	256 ± 94[a]	915 ± 343[a]	47.5 ± 15[d]

Islets were exposed to SZ and postcultured in different glucose concentrations as described in Table 4. The % of the controls was determined in each individual experiment by calculating the percentage of total insulin recovered from the SZ exposed islets compared with their corresponding controls. Values are means ± SEM for 4-5 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the corresponding control islets, postcultured at the same glucose concentrations, are: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$, using unpaired Student's t-test and [d] $p < 0.05$; [e] $p < 0.001$, using paired Student's t-test

The basal insulin release at 1.67 mmol/l glucose was higher in the control islets cultured at 28 mmol/l glucose compared with the other two groups of control islets ($p < 0.001$; analysis of variance) (Table 6). The control islets cultured at 11.1 or 28 mmol/l glucose showed a marked increase in their insulin output at 16.7 mmol/l glucose, whilst the control islets maintained at 5.6 mmol/l glucose barely responded to glucose. The rates of insulin secretion during stimulation with 16.7 mmol/l glucose in the islets exposed to SZ and cultured at 11.1 or 28 mmol/l glucose were decreased to about 20-25% of the corresponding control values. The insulin secretion at 16.7 mmol/l glucose of the islets treated with SZ and cultured at 5.6 mmol/l glucose was not different compared to the controls, probably as a consequence of the lack of insulin response in the controls. Among the groups of SZ treated islets, the islets cultured at 28 mmol/l glucose showed the highest insulin release rates at 16.7 mmol/l glucose ($p < 0.001$).

Discussion

The present study aimed to elucidate how glucose may affect islet SZ toxicity in vitro. Part of the study was thus performed by culturing islets at different glucose

Table 6. Glucose-stimulated insulin release in islets exposed to streptozotocin (SZ) and then postcultured at different glucose concentrations

Postculture glucose (mmol/l)	Treatment SZ (mmol/l)	Insulin release		
		1.67 mmol/l glucose (ng/10 islets × 60 min)	16.7 mmol/l glucose (ng/10 islets × 60 min)	% of the controls
5.6	0	0.4 ± 0.1	0.8 ± 0.2	-
5.6	1.8	0.9 ± 0.3	1.1 ± 0.1	ND
11.1	0	1.0 ± 0.4	25.0 ± 3.4	-
11.1	1.8	1.7 ± 0.7	5.5 ± 1.6[b]	21.7 ± 5.9[c]
28.0	0	12.6 ± 1.6	64.6 ± 6.4	-
28.0	1.8	3.8 ± 1.3[a]	14.6 ± 2.9[b]	24.3 ± 5.9[c]

Islets were exposed to SZ and postcultured in different glucose concentrations as described in Table 4. Islet insulin release was measured by incubating islets in groups of 10–14, as described in Materials and Methods. The % of the controls was determined in each individual experiment by calculating the percentage of insulin release at 16.7 mmol/l glucose of the SZ exposed groups compared with their controls. Values are means ± SEM of 6 experiments. Statistical significances of the observed differences between islets exposed to SZ versus the control islets, postcultured at the same glucose concentrations are: [a] $p < 0.01$; [b] $p < 0.001$, using unpaired Student's t-test and [c] $p < 0.001$, using paired Student's t-test. ND denotes not determined

concentrations before exposure to SZ. It is known [23] and also confirmed in this study (Tables 5 und 6) that islet culture in the RPMI 1640 medium supplemented with different glucose concentrations modifies the insulin content and the ability to release insulin after a glucose stimulation. It was therefore desirable to keep all the groups of islets in culture at the same glucose concentration (11.1 mmol/l glucose) after SZ exposure, to allow a valid comparison between the different groups. Furthermore, since recent findings suggest that SZ-induced damage to B cells in vitro is progressive [24, 25], the in vitro SZ cytotoxicity may be better evaluated after a period in culture following SZ treatment. Despite the difficulties outlined above, the role of the prevailing glucose concentration in the period after islet SZ exposure was also explored, since cellular events during the period after exposure to a toxin may be decisive for the survival of the islet cells [26].

In our first series of experiments islets previously maintained in a medium containing either 5.6 or 28 mmol/l glucose before exposure to SZ were less damaged by the drug than islets which had been cultured at 11.1 mmol/l glucose. The lowest sensitivity to SZ was found in the 28 mmol/l glucose group, as judged by the number of islets recovered, islet DNA and insulin content, and glucose-stimulated insulin release 3 days after SZ exposure. When the effects of different glucose concentrations in culture after the SZ treatment were studied, 28 mmol/l glucose also afforded a relative protection, mainly related to islet survival in comparison to islets maintained in either 5.6 or 11.1 mmol/l glucose.

The mechanism by which the damage to the islets cultured at 11.1 mmol/l glucose before SZ treatment was increased, in comparison with 5.6 mmol/l glucose, may be related to the functional activity of the B cells at the moment of SZ exposure. When mouse islets are cultured in RPMI 1640 medium, 11.1 mmol/l glucose seems to be a more appropriate glucose concentration than 5.6 mmol/l glucose for maintaining an intact B cell function. Islets cultured at that glucose concentration show higher insulin contents and insulin release after a glucose stimulation than islets cultured at 5.6 mmol/l glucose [23]. Moreover, the islets kept at 5.6 mmol/l glucose before SZ may also have benefitted from the rise in glucose concentration to 11.1 mmol/l glucose during the period of culture after SZ. Indeed, it has previously been reported that a rise in glucose concentration during a 20 h incubation period after SZ treatment may promote the survival of islet B cells [26].

Animals with chronic B-cell stimulation due to ventromedial hypothalamic lesions have an increased islet sensitivity to streptozotocin [27], whereas animals maintained on a carbohydrate-free, high protein diet, are resistant to the diabetogenic action of SZ [4]. Furthermore, acute in vivo administration of phentolamine [28–30] or yohimbine [30], which stimulate insulin release in vivo [12], increase the susceptibility of B cells to damage by SZ. These observations are supported by previous in vivo findings, suggesting that glucose injections can potentiate the diabetogenic action of SZ [9–12]. This later finding may reflect an increase in the functional activity of B cells at the moment of SZ exposure, making them more vulnerable to the toxin. Previous in vitro observations concerning glucose and SZ are difficult to reconcile with the current data, since these studies dealt only with acute preincubations with glucose and subsequent measurements of insulin biosynthesis or release immediately after SZ exposure [13–14]. The present finding of an increased sensitivity to SZ of islets maintained at 11.1 mmol/l glucose compared to 5.6 or 28 mmol/l glucose, have also been observed after exposure of B cells to interleukin-1 [31, 32], a cytokine with a suggested role in the pathogenesis of human Type 1 (insulin-dependent) diabetes mellitus.

If a higher metabolic and secretory activity of the B cells sensitises them to the cytotoxic action of SZ, it is somewhat difficult to explain the present finding that islets cultured at 28 mmol/l glucose before SZ treatment retained a better functional capacity than is-

lets precultured in 5.6 or 11.1 mmol/l glucose. It may be that we are faced with a different effect of glucose when added to the culture medium at a very high concentration, i.e. an activation of the defense mechanisms of the B cells against toxins. Indeed, Pipeleers and Van de Winkel [26] showed that B cells incubated in a high glucose medium (20 mmol/l), after exposure to four different B-cytotoxic agents (streptozotocin, alloxan, t-butylhydroperoxide and B cell surface antibodies plus complement) were able to partially counteract the damaging effects of all these cytotoxins. Culture in a high glucose medium (28 mmol/l glucose) has also been found to increase the functional survival of islets subjected to minor intracellular injuries following cryopreservation [23]. The present study is in line with these findings since culture of islets at 28 mmol/l glucose after SZ increased the survival of islet cells. It is notable that the culture at 28 mmol/l glucose after SZ mainly protected the islets in terms of survival, whereas the functional activity of the B cells, as measured by glucose-stimulated insulin release, remained inhibited. This suggests that the high glucose environment essentially protected the B cells against death induced by SZ, but was ineffective to counteract the long lasting impairment in insulin production in the surviving B cells induced by the toxin [33].

The molecular mechanism behind the protective effect of high glucose culture on B cells still remains elusive. The observation that it may occur with such different B-cytotoxins as interleukin 1, alloxan, streptozotocin and t-butylhydroperoxide, suggests that we are dealing with a broader phenomenon than just a transitory interference with a chemical cytotoxin. One possibility is that a high glucose concentration facilitates islet ATP production required for different energy-dependent processes operative during the critical period of cellular repair after exposure to various noxious agents.

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